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# Spatial and memory circuits in the medial entorhinal cortex

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The large capacity of episodic memory is thought to be supported by the emergence of distinct hippocampal cell assemblies for unrelated memories, such that interference is minimized. In large-scale population recordings, the orthogonal nature of hippocampal representations across environments is evident in the complete reorganization of the firing locations of hippocampal place cells. Entorhinal grid cells provide inputs to the hippocampus, and their firing patterns shift relative to each other across different environments. Although this suggests that altered grid cell firing could generate distinct hippocampal population codes, it has recently been shown that new and distinct hippocampal place fields emerge while grid cell firing is compromised. We therefore propose that separate circuits within the medial entorhinal cortex are specialized for performing either spatial or memory-related computations.

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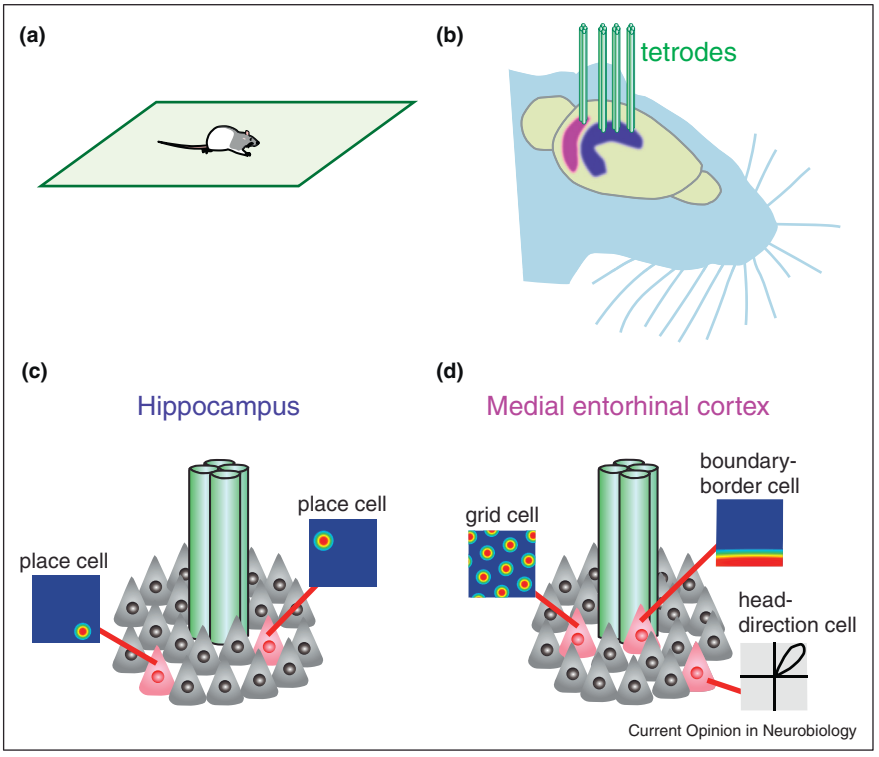
## Introduction

Episodic memories rely on complex neural processes, not only because they require long-term storage for events that occur just once, but also because each experience needs to be stored distinctly from similar ones. There is a general consensus that the medial temporal lobe, which includes entorhino-hippocampal circuitry, is critical for long-term episodic memory in both humans and animals [1,2], and that neuronal networks within these structures are specialized to meet the requirements for memory processing. For example, hippocampal cell populations jointly represent many features of an event, including space, context, and time [3] and perform network computations to distinctly code these features for each event. The emergence of the combinatorial representation of many aspects of an experience in hippocampal networks is thought to be based on convergent anatomical connections [4]. Sensory information is first processed in separate streams throughout a number of cortical areas, begins to converge in cortical association areas, is further integrated in the projections from association areas to the entorhinal cortex, and finally in the projection from entorhinal subdivisions to the hippocampus [5]. The pathway through the medial entorhinal cortex (MEC) appears specialized for computing and conveying spatial information to the hippocampus while the pathway through the lateral entorhinal cortex (LEC) appears specialized in conveying object and object/place-related information [3]. While functional cell types and computations in LEC have only been described to a limited extent, much additional information about cell types and putative computations in MEC has emerged over the past ten years. In particular, multi-electrode recording techniques that allow for the simultaneous recording of dozens of neurons in behaving subjects have substantially advanced our understanding of how the changes in entorhinal firing patterns could result in the distinct hippocampal coding for different environments. In this review we will briefly summarize our current understanding of the connectivity of functionally and anatomically identified cell types in MEC. We will then critically examine the view that one of the main functions of the MEC is to forward highly distinct spatial firing patterns to the hippocampus. Based on recent findings, it is suggested that the emergence of distinct spatial maps in hippocampus does not require inputs from grid cells in MEC and that spatial maps are even partially preserved without any MEC input.

## Which spatial signals from MEC are forwarded to the hippocampus?

Consistent with the anatomical position of MEC in receiving information from cortical areas that process spatial information [6,7] numerous cell types that exhibit spatial and/or directional firing patterns have been described in the cell layers of MEC (see Figure 1). The spatial tuning of each cell type has been shown to be aligned in a particular way to the environment. Head direction (HD) cells are aligned to compass directions, and each HD cell fires at high rates when the head is oriented in a particular angular position in the horizontal plane [8]. HD cells are found throughout most layers of

**Figure 1**




Large-scale electrophysiological recordings in awake-behaving rodents has revealed distinct cells types and network computations in the medial entorhinal cortex and hippocampus. **(a)** While rats explore an environment the activity patterns of populations of neurons can be monitored using large-scale recording techniques. **(b)** Recording arrays with multiple independently moveable electrode bundles that each consist of four electrodes (*i.e.*, tetrodes) allow for the sampling of large brain regions within the rodent brain. Here, the medial entorhinal cortex (MEC) is shown in purple and the hippocampus in blue. **(c)** and **(d)** The four electrodes that comprise a tetrode can record the action potentials of dozens of neurons located in close proximity to the electrode tip. This method, used in awake-behaving rats, has revealed distinct functional cell types in MEC and in hippocampus and has been critical for determining network computations by simultaneously monitoring the activity patterns of a large number of neurons. (c) Hippocampal principal neurons are spatially tuned and fire action potentials at distinct locations in an environment that an animal actively explores (see (a)). Firing rate maps are shown for two active place cells (highlighted in pink). For rate maps, peak rates are indicated in red, zero firing in blue. (d) Grid cells, border cells, and head-direction cells comprise a large fraction of the cells in MEC.

MEC and are thought to receive their information from the presubiculum, where this cell type is particularly abundant. Another cell type that is found throughout all layers of MEC as well as in connected cortical areas, such as the subiculum, presubiculum, and parasubiculum, are boundary/border cells [9,10]. Boundary/border cells are aligned to prominent borders and fire either directly at the border or at a set distance from a border. Although distributed throughout many of the same regions that contain HD cells, boundary cells comprise a much smaller fraction of the population. A third specialized cell type are grid cells. They are most numerous in the superficial layers (II and III) of MEC, which send direct inputs to all hippocampal subregions, but are also found in the deep MEC layers and in the presubiculum and parasubiculum. Each grid cell has multiple firing fields that are organized in a periodic hexagonal lattice across the surface of an environment [11]. For a given grid cell, the spacing between grid peaks is fixed, and grid cells with similar spacing are found in patches. These patches are arranged so that grid cells with smaller spacing are found in more dorsal patches and grid cells with larger spacing in more ventral patches [11,12•]. As revealed from large-scale population recordings, this organization is accompanied by discrete jumps in grid spacing along the dorsoventral axis of MEC such that there is modular organization in grid size. When rats are moved from one environment to another, cells within each module display coherent shifts and rotations with respect to each other, while different modules show discordant shifts and rotations. The characterization of grid cells in the MEC of rodents and other species [11,13,14] has raised several hypotheses that center on the notion that entorhinal grid cells are specialized for path integration and navigation [15] and are, while these computations are performed, the primary source for updating spatial information in hippocampal place cells.

Based on the hypothesis that the highly precise spatial representation of grid cells rather than of other cell types serves as a primary source for hippocampal spatial firing,

anatomical studies have sought to describe the detailed connectivity patterns between various cell types in the superficial layers of MEC and hippocampal place cells. MEC layer III consists of principal cells that project to CA1 and subiculum [4], while layer II consists of two distinct principal cell populations with different projection targets. First, calbindin-positive layer II cells are clustered in patches and are anatomically arranged in a hexagonal grid pattern [16•,17•]. This cell type is mostly composed of pyramidal cells that appear to only project sparsely to select hippocampal subregions [16•,17•,18]. Second, reelin-positive stellate cells widely project to the hippocampus and give rise to the perforant pathway targeting the dentate gyrus and CA3. Does either of these anatomically defined cell types correspond to grid cells? Previous studies using *in vivo* patch-clamp recording from head-fixed mice have reported that the majority of layer II grid cells have stellate-specific morphology and intrinsic conductances [19••,20••]. In contrast, a recent study using juxtacellular recordings identified that theta rhythmicity, which is a typical feature of grid cell discharge, is stronger in pyramidal compared to stellate cells [16•]. Furthermore, calbindin-positive patches were identified to be selectively targeted by cholinergic neurons from the medial septal area (MS). Because grid firing in rodents is sensitive to MS inactivation [21,22••], this would suggest a stronger association between grid cells and pyramidal cells or, at least, that the emergence of grid patterns requires this cell type. In summary, the existing data have identified each of the two anatomical cell classes as putative grid cells and are consistent with the notion that there is no strict correspondence between functional and anatomically identified cell types.

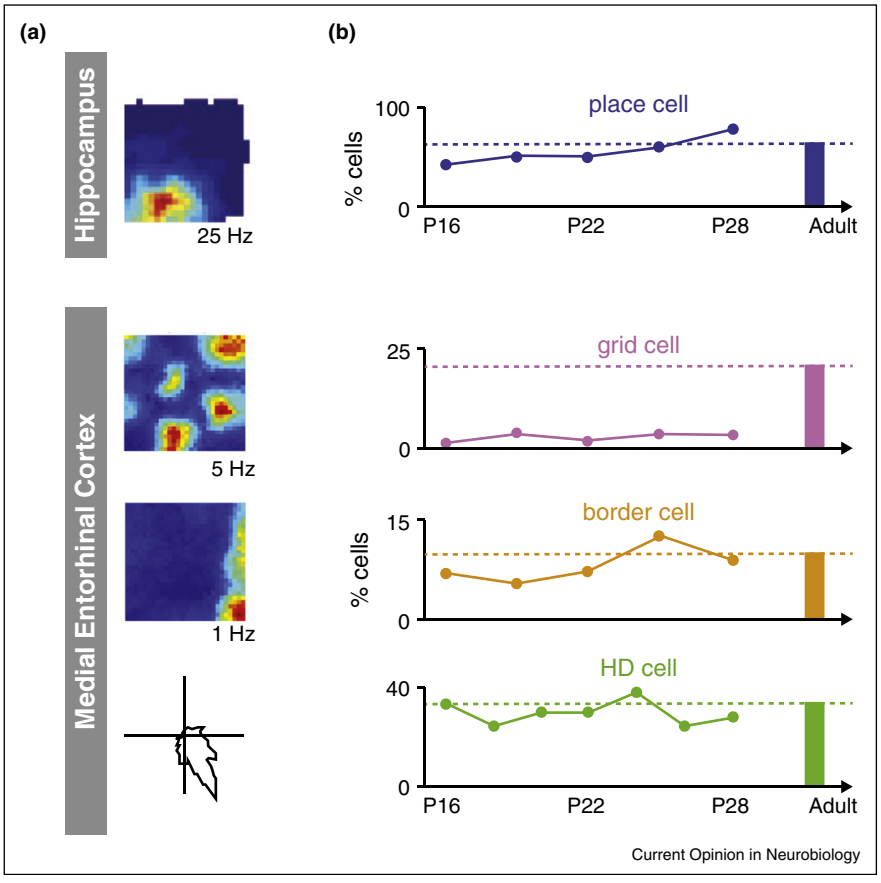
Because there is no direct match between anatomically and functionally identified cell types, a recent study combined optogenetic labelling of synaptic partners with electrophysiological recordings in the MEC to address the question whether grid cells project to the hippocampus. This study confirmed that neurons with periodic grid firing patterns in the MEC form direct excitatory projections to the hippocampus. However, the fraction of grid cells was found to be relatively low, making up only ~30% of the total hippocampus-projecting cell population in the MEC [23••]. The remaining MEC inputs to the hippocampus were from boundary/border cells, from HD cells, and from a large fraction (~50%) of cells without any measureable spatial selectivity. In addition to grid cells, many other cell types therefore have prominent connections to the hippocampus.

The projections from the additional entorhinal cell types to the hippocampus suggest that these cells have important roles in maintaining hippocampal firing patterns. This possibility is supported by recent findings that grid cells are the last spatially tuned cell type to emerge in MEC during development, well after the emergence of place cells in hippocampus, and boundary/border cells and HD cells in the entorhinal cortex (Figure 2b) [24,25,26••]. These developmental time courses demonstrate that hippocampal place cells can exist before receiving inputs from strongly tuned grid patterns. It is nonetheless possible that grid cells have a major role in sustaining hippocampal firing patterns once they have developed, and then provide the most prominent spatial inputs to the hippocampus. This can be tested by using MS inactivation, which selectively abolishes the spatial firing patterns of grid cells while largely sparing the spatial firing of boundary/border cells and the direction tuning of HD cells (Figure 3a) [21,22••,27••]. Using this method in combination with large-scale population recording, it has been shown that hippocampal place cells were preserved in familiar environments [22••] (Figure 2b) and that the preserved place fields could even sharpen their location-specific firing pattern [27••]. Therefore, the persistence of hippocampal spatial maps in familiar environments does not depend on MEC grid cells. This raises the question whether the primary role of grid cells is to generate distinct hippocampal maps rather than to sustaining already established hippocampal maps.

## Are grid cells required for new orthogonal spatial maps in hippocampus?

Mechanisms that increase the number of distinct population codes in hippocampal networks increase the number of memories that can potentially be stored without interference from previously stored memories. Because neuronal activity in hippocampus is organized into place fields, differences in population coding can be measured by determining the similarity of spatial firing across environments, and distinct spatial firing patterns are commonly referred to as 'remapping' [28,29]. Remapping can be generated in the hippocampus by either changing the firing rates within the currently active subset of neurons or by activating different subsets of neurons. These different types of remapping can best be distinguished by monitoring the activity patterns of large populations of neurons. Using these methods, it was found, for changes in the environment at the same spatial location, that place cells modify the intensity of firing within the place field, which is referred to as rate remapping [30]. Rate remapping was shown to depend on intrahippocampal computations [31] and on inputs from LEC [32•], while MEC grid cell firing patterns remain unchanged in this condition [33]. On the other hand, when moving to a different environment, place cells can turn on, turn off, and fire with a new relation to each other, such that their spatial firing patterns become completely unrelated, which is referred to as global remapping [30]. Global remapping is accompanied by shifts in the firing phase of MEC grid cells [33], where grid cells in one module shift and rotate together while grid cells in different modules shift and rotate with respect to each other [12•]. Because grid cells project to the hippocampus, the

**Figure 2**

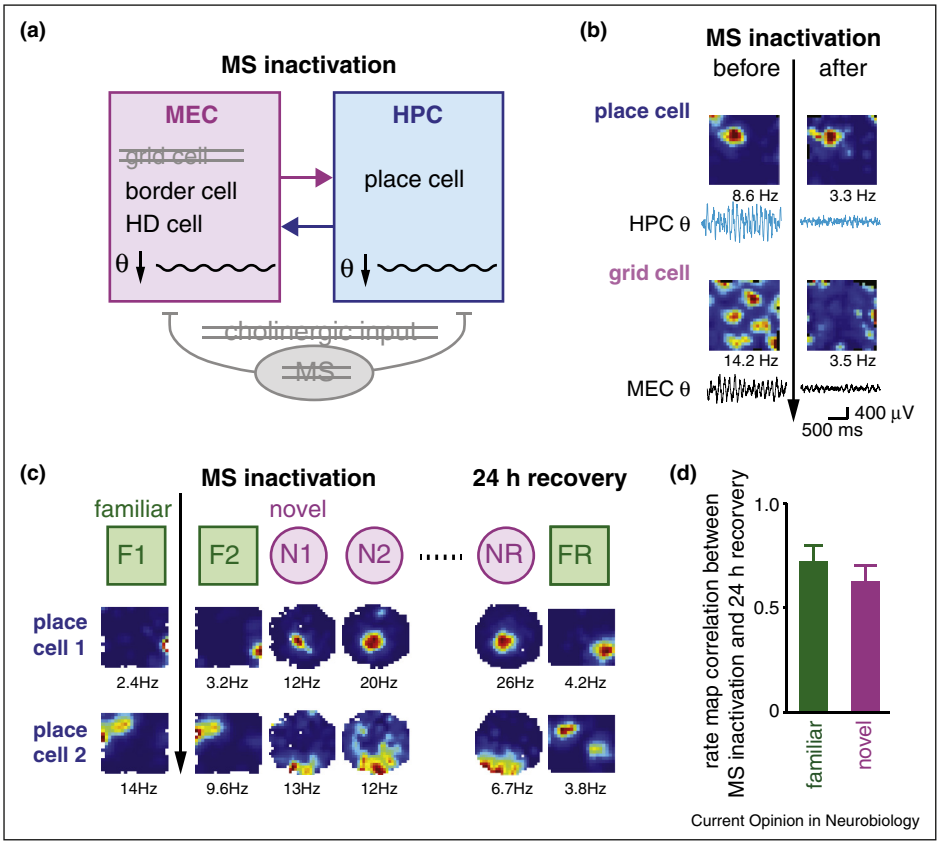



Place fields are present in development prior to grid cells. **(a)** Representative firing rate maps are shown for spatially tuned cells in hippocampus and entorhinal cortex, and a polar plot is shown for a directionally tuned cell. For rate maps, peak rates are indicated in red, zero firing in blue. From top to bottom: hippocampal place cell, MEC grid cell, MEC boundary/border cell, and MEC head-direction cell. **(b)** The proportion of each functionally distinct cell type as a function of age. Hippocampal place cells, MEC head-direction (HD) cells, and boundary/border cells express adult-like firing fields when rat pups are first exposed to an open field at the age of 2.5 weeks, whereas grid cell firing patterns progressively emerge and are refined until the age of four weeks.
Adapted from Ref. [24,25,26••].

reorganization in grid firing patterns was proposed to cause global remapping in the hippocampus [34,35].

If the orthogonalization in hippocampus were to rely on shifts in grid cell modules, it would be expected that the silencing of grid cell networks in MEC would interfere with the development of independent maps for different spatial locations. Brandon *et al.* [27••] recently examined this hypothesis by recording from hippocampal networks in novel and familiar environments while manipulating the periodic firing of grid cells. MS inactivation, which has been shown to reliably disrupt the spatial firing patterns of grid cells [21,22••] was used to selectively silence the grid cell network, while sparing the spatial tuning of other MEC cell types such as HD cells and boundary/border cells. The testing in novel environments during MS inactivation showed that new place fields formed without grid cell input (Figure 3c). Furthermore, the new fields remained stable when returning to the same environment multiple times, and they were entirely distinct from those in the familiar room. This finding suggests that grid cells and shifts of grid patterns are not necessary for global remapping to emerge. In further support of the conclusion that grid cells do not directly result in remapping, it was also shown that place fields that were generated during the inactivation were not altered after recovery from the inactivation (Figure 3d). The reemergence of regular grid firing does therefore not alter firing patterns that were originally established with disrupted grid cell activity. These results challenge models in which spatial maps and orthogonal spatial representations in hippocampal neural networks rely on grid cell input (Figure 4) [12•,33–38]. In addition, the MS inactivation is also accompanied by a loss of cholinergic input to hippocampus and

**Figure 3**



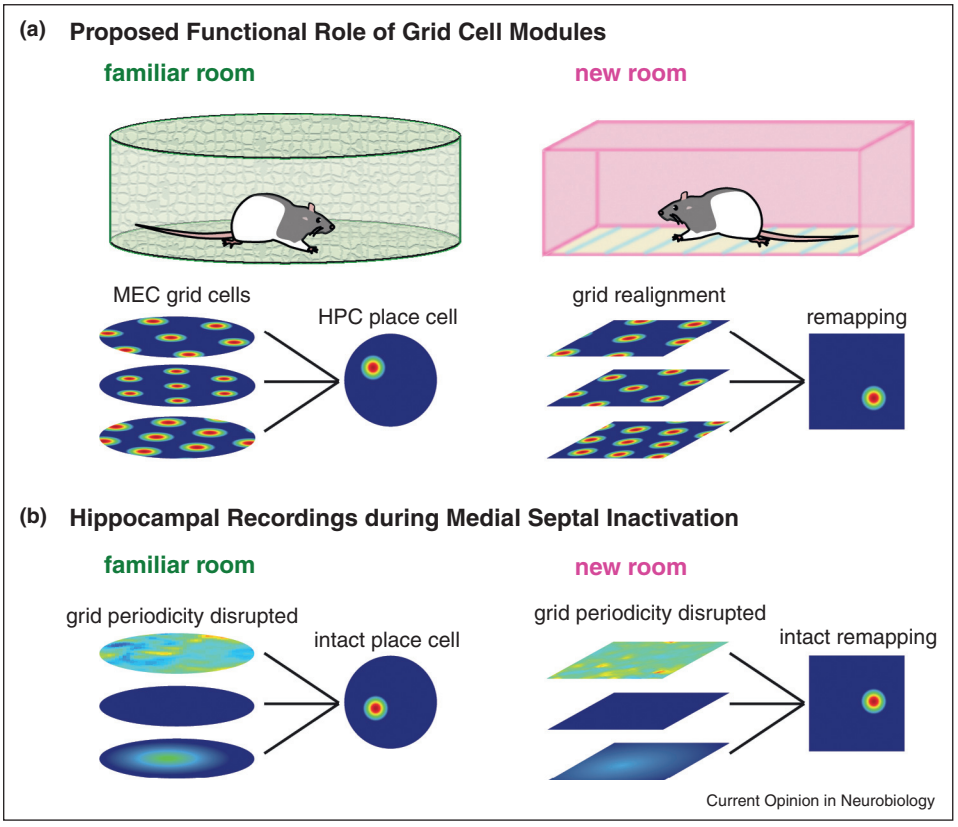

Distinct spatial firing patterns in hippocampus develop without grid cell input. **(a)** Schematic of the effect of medial septal (MS) inactivation on medial entorhinal cortex (MEC) and hippocampal function. Grid cells in MEC are selectively silenced (gray text) while boundary/border cells and head direction cells remain largely unchanged (black text). Place fields in hippocampus are retained. The local theta oscillation in both regions is reduced substantially and proportionally. Cholinergic input is also blocked. **(b)** The activity pattern of a representative place cell and a representative grid cell before and after MS inactivation with lidocaine. Spatial firing is preserved in the hippocampal place cell while periodic firing is abolished in the MEC grid cell. Theta power was substantially reduced in both the MEC and the hippocampus (Adapted from Ref. [22••]). **(c)** Stable hippocampal place fields can emerge in a novel environment during MS inactivation by muscimol infusion (N1,N2). Newly formed place fields are retained when grid cell firing has recovered (NR). **(d)** Spatial maps were unchanged when grid cell activity reemerged at recovery, as indicated by similar rate map correlations between the first session in the novel environment and retesting at 24 h after the inactivation. Adapted from Ref. [27••].

entorhinal cortex, as well as a significant reduction in the theta oscillation. Therefore, hippocampal remapping is also independent of these dynamic regulators, which are known to significantly influence hippocampal memory processing [39,40].

## Transformation of spatial signals from MEC to hippocampus

The anatomical and functional evidence that is summarized above suggests that spatial signals from multiple cell types in MEC or from other parallel pathways, such as the lateral division of the entorhinal cortex, might have redundant roles in sustaining a robust spatial representation in hippocampus [41,42]. HD cells and boundary-border cells have both been identified as projecting directly from MEC to the dorsal hippocampus [23••] and may thus provide spatial information to place cells in parallel to grid cells. Such a possibility had already been suggested by models of place cell formation and remapping that were predominantly based on spatial signals from boundary cells to the hippocampus [43–45]. Moreover, it has recently been found that LEC neurons do not only robustly code for object identity [46] but can also represent past spatial locations of known objects [47]. Spatial information could thus also be available to hippocampal circuits through inputs arriving from LEC. The extent to which spatial coding in hippocampus depends predominantly on the spatially tuned cells in MEC or could be complemented by LEC inputs can be tested by recording from hippocampus after lesions that are targeted to the MEC. While earlier lesions of the MEC may have spared grid cell areas [48], Hales *et al.* [49•] recently

**Figure 4**




Hippocampal orthogonalization can occur without input from MEC grid cells. **(a)** The shift in grid cell modules between distinct spatial locations has been hypothesized to drive the reorganization of hippocampal representations such that independent maps for distinct locations are formed. **(b)** MS inactivation is currently the only known manipulation that results in the disruption of grid cell activity while leaving the firing properties of other spatially tuned cells in MEC largely unchanged. Using this technique, it has been shown that grid cells become silent and lose their periodic spatial firing patterns. Despite the disruption of grid firing, the spatial firing of established hippocampal place cells are unaltered, new place fields are formed in novel environments, and independent spatial maps are established for different environments [27••]. Experimental evidence therefore suggests that grid cell inputs to the hippocampus are not necessary for hippocampal spatial map formation and the orthogonalization of firing patterns.

showed that a complete MEC lesion that included grid cell areas resulted in a substantial broadening of hippocampal place fields, but nonetheless in a partial sparing of spatial coding in the hippocampus. Notably, effects on place field size were observed after MEC lesions and not after MS inactivation, which suggest that precise spatial information in hippocampus requires inputs from MEC cells but not necessarily from MEC grid cells.

Taken together, these studies suggest that the generation of spatial maps may be a widely distributed function of the brain, and that grid cells may have a different function than continuously updating hippocampal spatial coding. These findings are consistent with proposing a more prominent role of entorhinal cortex for memory rather than spatial coding [34,50] or with the possibility that the entorhinal cortex supports spatial coding and path integration without continuously relying on its connection with the hippocampus [51]. The view that entorhinal spatial processing can be performed independent of the hippocampus has been challenged by the finding that entorhinal grid cell firing is disrupted during hippocampal inactivation [52•]. However, hippocampal inactivation was found to not only immediately shut down hippocampal neuronal activity but also to gradually reduce the amplitude of the local theta oscillation in MEC. The gradual effect on theta oscillations paralleled the time course of the reduction in grid firing and reached levels known to result in disrupted periodic firing in grid cells [21,22••,27••]. Furthermore, the gradual effect on theta and grid cells resulted in a period during which the hippocampus was inactive while theta and grid cell firing was not yet substantially reduced [51]. It is therefore plausible that disrupted grid cells are not a direct effect of the reduced neuronal activity in the hippocampus and that spatial processing in entorhinal

cortex can be performed independent of hippocampal feedback.

## Conclusions

Recent anatomical studies have found different projection patterns for pyramidal and stellate cells in MEC layer II, and large-scale population recording in hippocampus revealed that orthogonalized hippocampal representations emerged without grid cell inputs. Based on these findings we hypothesize that memory and spatial functions are supported by two distinct local MEC circuits, which are each preferentially associated with one of the two anatomically distinct cell classes in layer II. Entorhinal pyramidal cells are suggested to be more specialized for intra-entorhinal computations, including spatial periodicity and path integration, while stellate cells are suggested to support memory-related firing patterns throughout the entorhino-hippocampal loop including orthogonalization and phase precession. Grid firing patterns have been reported in pyramidal and stellate cells and could thus perform specialized functions dependent on the circuit in which they are integrated. Future large-scale electrophysiological studies, coupled with refined anatomical and genetic manipulations, are needed to provide direct evidence of separate roles of grid cells in intraentorhinal spatial processing and in memory processing across the entorhino-hippocampal loop.

## Conflict of interest statement

Nothing declared.

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